

## Cytological Studies of the Genus *Carex* (Cyperaceae) in the Osumi Islands (Kagoshima Prefecture) II. Chromosome Counts of Four Species Collected from Kuroshima Island

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Four species of the genus *Carex* from Kuroshima Island were used for karyomorphological studies. Intraspecific aneuploidy,  $2n = 38 = 19\text{II}$ ,  $39 = 19\text{II} + \text{I}$ , and 58, were found in *C. atroviridis* var. *scabrocaudata* and these chromosome numbers are reported for the first time in this study. Chromosome numbers of *C. multiflora* var. *pallidisquama* ( $2n = 72$ ), *C. tokarensis* ( $2n = 26 = 13\text{II}$ ), and *C. tsushimensis* ( $2n = 32 = 16\text{II}$ ) were determined for the first time. *Carex atroviridis* var. *scabrocaudata* and *C. tokarensis* are endemic to the Kuroshima and Tokara Islands. Our results suggest a close relationship among *C. atroviridis* var. *scabrocaudata*, *C. conica*, and *C. oshimensis*. *Carex tokarensis* is also considered to be closely related to *C. reinii*, because these two species had the same chromosome number.

**Key words:** *Carex*, chromosome number, Cyperaceae, intraspecific aneuploidy, Kuroshima Island.

Kuroshima Island belongs to the Osumi Island group, and is located about 55 km southwest of the Satsuma Peninsula in Kagoshima Prefecture, Japan (30°N, 129°E). Sako and Maruno (1983) reported that the northern-most or southern-most distributions of many vascular plants are found on this island, including representatives of the genus *Carex*. There are more than 200 *Carex* species in Japan (Ohwi 1936, Akiyama 1955, Koyama 1962, Katsuyama 2005), and 11 species have been reported from the Kuroshima Island (Sako and Maruno 1983). *Carex atroviridis* var. *scabrocaudata* and *C. tokarensis* are endemic to the Kuroshima and Tokara Islands (Katsuyama 2005), and Kuroshima Island is the northern limit of both these species (Sako and Maruno 1983).

The chromosome numbers of Japanese species of *Carex* have been reported by Tanaka (1948), Hoshino (1981, 1992), Hoshino and Okamura (1994), and Hoshino and Waterway (1994). They also reported the existence of extensive interspecific and intraspecific aneuploidy. However, there have been no published reports of cytological studies of the genus *Carex* from Kuroshima Island. The purpose of this paper is to report the chromosome numbers of four species of the genus *Carex* from Kuroshima Island, and to discuss their relationships to allied species.

### Materials and Methods

Materials collected from four species of the genus *Carex* from Kuroshima Island, all

Table 1. Species, localities, and voucher specimens, and chromosome numbers of four species of the genus *Carex* collected from Kuroshima Island in Kagoshima Prefecture, Japan

Species	Locality and Voucher specimen	Chromosome number; 2n (n)
<i>Carex atroviridis</i> Ohwi var. <i>scabrocaudata</i> T. Koyama		
Mishima, Nakasato; Hoshino & al. 19395 (OKAY)		38 (19II)
Mishima, Mt. Yagura; Hoshino & al. 19398 (OKAY)		39 (19II+I)
Mishima, Mt. Yagura; Hoshino & al. 19399 (OKAY)		38
Mishima, Mt. Yagura; Hoshino & al. 19401 (OKAY)		58
<i>C. multiflora</i> Ohwi var. <i>pallidisquama</i> Ohwi		
Mishima, Mt. Yagura; Hoshino & al. 19400 (OKAY)		72
<i>C. tokarensis</i> T. Koyama		
Mishima, Nakasato; Hoshino & al. 19393 (OKAY)		26 (13II)
Mishima, Mt. Kamuko to west valley; Hoshino & al. 19418 (OKAY)		26
<i>C. tsushimensis</i> (Ohwi) Ohwi		
Mishima, Nakasato, Inokuchi river-side; Hoshino & al. 19422 (OKAY)		32 (16II)

endemic to Japan (Katsuyama 2005), were used for karyomorphological observations. The materials examined are listed in Table 1. Somatic chromosomes were observed in the meristematic cells of root tips. The root tips were pretreated in 0.002 M 8-hydroxyquinoline solution for 1 h at 23°C and then 15 h at 4°C. They were then fixed in acetic alcohol (1:3) for at least 16 h at -20°C or for 1.5 h at 23°C, stained using Feulgen's nuclear reaction, macerated in a mixture of 2 % pectinase and 2 % cellulase for 1 h at 37°C, restained in 1 % aceto-orcein, and then squashed. Meiotic chromosomes were also observed in pollen mother cells. Spikelets were fixed in acetic alcohol (1:3) for at least 6 h at -20°C. Anthers were stained in 1 % aceto-orcein and then squashed. Voucher specimens are deposited in the Herbarium of Okayama University of Science (OKAY).

### Results and Discussion

The chromosome numbers determined in this study are shown in Table 1. *Carex atroviridis* var. *scabrocaudata* had intraspecific aneuploidy,  $2n = 38 = 19II$ ,  $39 = 19II + I$ , and 58, and these chromosome numbers are reported here for the first time. Somatic metaphase chromosomes ranged from 0.9 to 1.9  $\mu m$  in length (Fig. 1A–C).

The  $2n = 38$  plant had the normal 19 bivalents in meiotic division and the length of meiotic metaphase chromosomes ranged from 1.0 to 1.8  $\mu m$  (Fig. 2A). The  $2n = 39$  plant had 19 bivalents and one univalent in meiotic division. The 19 bivalent chromosomes ranged from 1.0 to 1.5  $\mu m$  in length and the one univalent chromosome was less than 0.5  $\mu m$  in length (Fig. 2B). Intraspecific aneuploids with many irregular meiosis configurations have been reported in *C. blepharicarpa*, *C. conica*, *C. duvaliana*, and *C. stenostachys* (Hoshino et al. 1993, Hoshino and Okamura 1994, Hoshino and Onimatsu 1994, Hoshino and Waterway 1994). These authors suggested that these aneuploids with many irregular meiotic configurations originated from chromosome fission or fusion. In the present study, intraspecific aneuploids of *C. atroviridis* var. *scabrocaudata*,  $2n = 38$ , 39, and 58, were found in the same population. The  $2n = 39$  plant had one small univalent chromosome, which might have originated from chromosome fission. The  $2n = 58$  plant may have quite a different origin. It could have originated from fusion of an unreduced gamete of  $2n = 39$  and a reduced gamete of  $2n = 38$  (i. e.,  $2n = 58 = 39 + 19$ ). Further studies should involve examination of the meiotic

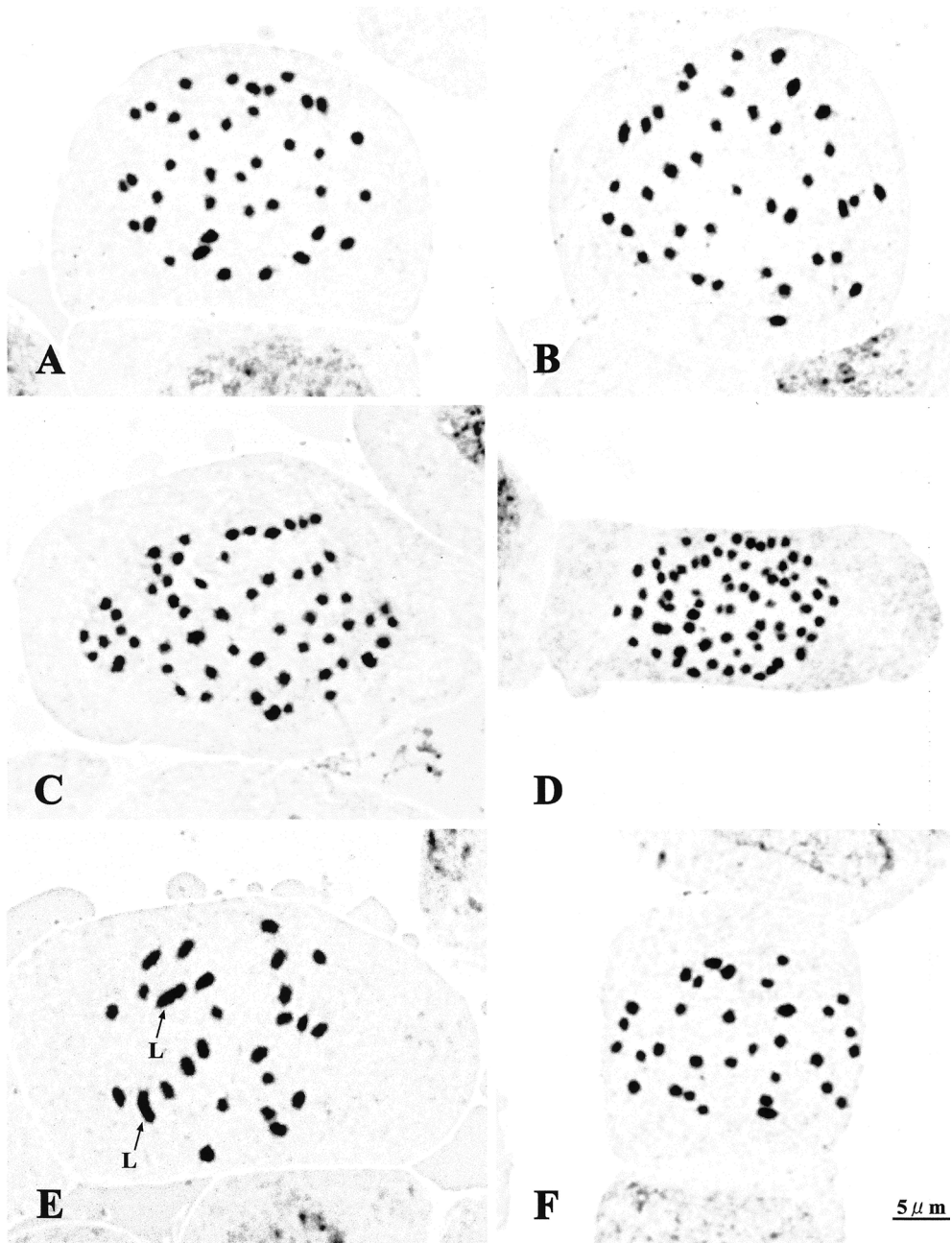


Fig. 1. Photomicrographs of somatic metaphase chromosomes of four species of the genus *Carex* from the Kuroshima Island. A, B, C. *C. atroviridis* var. *scabrocaudata* ( $2n = 38, 39$ , and  $58$ ). D. *C. multiflora* var. *pallidisquama* ( $2n = 72$ ). E. *C. tokarensis* ( $2n = 26$ ). F. *C. tsushimensis* ( $2n = 32$ ). Arrows indicate two large chromosomes (L).

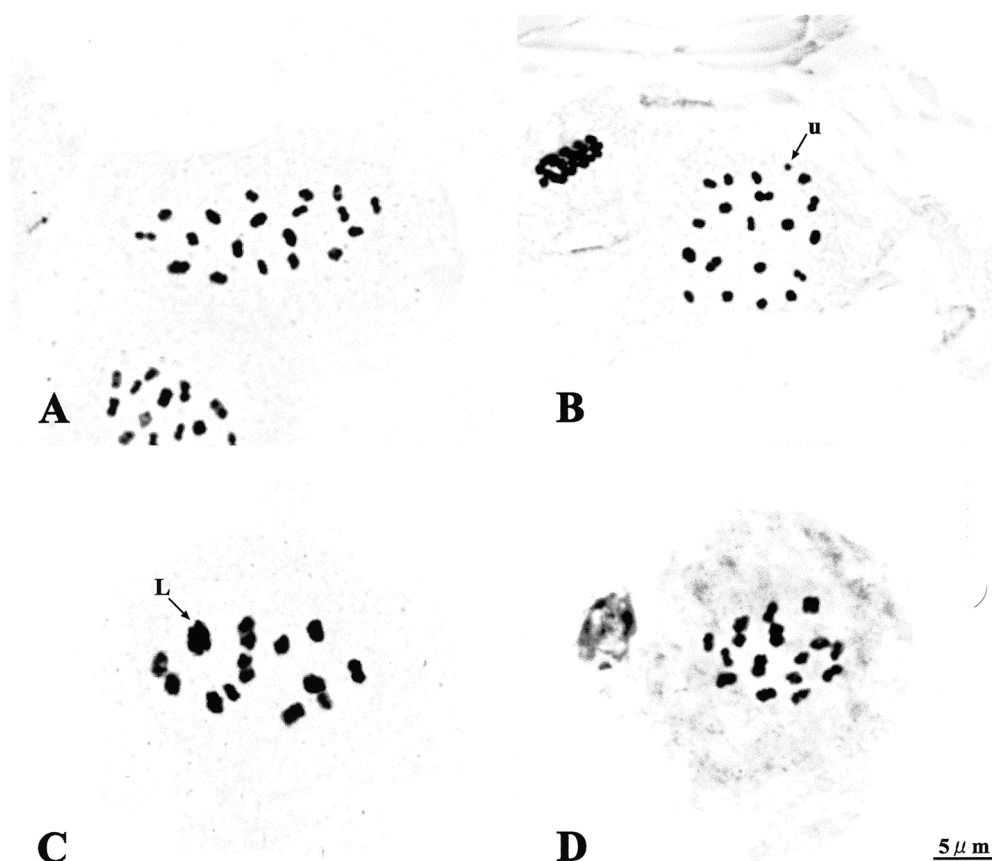


Fig. 2. Photomicrographs of meiotic metaphase I chromosomes of three species of the genus *Carex* from the Kuroshima Island. A, B. *C. atroviridis* var. *scabrocaudata* ( $2n = 38 = 19\text{II}$  and  $39 = 19\text{II} + \text{I}$ ). C. *C. tokarensis* ( $2n = 26 = 13\text{II}$ ). D. *C. tsushimensis* ( $2n = 32 = 16\text{II}$ ). Arrows indicate univalent (u) and large bivalent (L) chromosomes.

configurations of more samples in order to clarify the origins of intraspecific aneuploids in this species.

*Carex atroviridis* var. *scabrocaudata* was described from Nakanoshima Island of Tokara Islands by Koyama (1957). Hatusima (1986) and Katsuyama (2005) considered that this variety is closely related to *C. conica* used a combination *C. conica* var. *scabrocaudata* but this combination has not been valid by published. Morphologically this variety, *C. conica* and *C. oshimensis*, share dark-brown staminate spikes and elliptical achenes. *Carex atroviridis* var. *scabro-*

*caudata* differs from *C. atroviridis* in cylindrical pistillate spikes with dense perigynia. The chromosome number of *C. atroviridis* was  $2n = 70$  (Yano et al. unpublished). The chromosome number of *C. conica* was reported as being  $2n = 34, 35$ , and  $38$  by Tanaka (1948). Hoshino and Waterway (1994) also reported  $2n = 32, 33, 34, 36, 37$ , and  $38$  for *C. conica*. Tanaka (1948) reported  $2n = 34$  and  $38$  for *C. oshimensis*. Our results show a close relationship between *C. atroviridis* var. *scabrocaudata*, *C. conica*, and *C. oshimensis*, but not of *C. atroviridis* to them. New combination for this variety

will be proposed in another report.

*Carex multiflora* var. *pallidisquama* had a chromosome number of  $2n = 72$ , the first number to be determined for this species. Somatic metaphase chromosomes ranged from 0.6 to 1.3  $\mu\text{m}$  in length (Fig. 1D). Katsuyama (2005) reported that *C. multiflora* var. *pallidisquama* is closely related to *C. multiflora* var. *multiflora*. The chromosome number of *C. multiflora* var. *multiflora* was reported to be  $2n = 70$  by Hoshino (1981). Our results support the close relationship between these two varieties.

*Carex tokarensis* had the chromosome number of  $2n = 26 = 13\text{II}$ , the first number to be determined for this species. The  $2n = 26$  chromosomes showed a bimodal karyotype, with two large and 24 small chromosomes (Figs. 1E, 2C). In somatic metaphase chromosomes, the two large chromosomes were larger than 2.8  $\mu\text{m}$ , and the 24 small chromosomes ranged from 1.1 to 2.0  $\mu\text{m}$  in length (Fig. 1E). *Carex tokarensis* had 13 normal bivalents pairing in meiotic division. In meiotic metaphase chromosomes, the large chromosomes were larger than 3.0  $\mu\text{m}$  and the small chromosomes ranged from 1.5 to 2.5  $\mu\text{m}$  in length (Fig. 2C). Katsuyama (2005) assigned *C. tokarensis* to section *Decorae* together with *C. reinii*. The chromosome number of *C. reinii* was reported as being  $2n = 26$  by Tanaka (1948). Hoshino (1981) also reported  $2n = 25$  and 26 for *C. reinii*. Our results confirm the close relationship between *C. tokarensis* and *C. reinii*.

*Carex tsushimensis* had the chromosome number of  $2n = 32 = 16\text{II}$ , the first number to be determined for this species. Somatic metaphase chromosomes ranged from 1.0 to 1.6  $\mu\text{m}$  in length (Fig. 1F). *Carex tsushimensis* had 16 normal bivalents pairing in meiotic division. Meiotic metaphase chromosomes ranged from 1.1 to 1.6  $\mu\text{m}$  in length (Fig. 2D). Koyama (1962) recognized *C. tsushimensis* as a variety of *C. sociata*. Katsuyama (2005) reported that *C.*

*tsushimensis* is closely related to *C. sociata* and *C. uber*, sharing pale-green staminate spikes and rhombic achenes. Ohkawa et al. (2000) reported  $2n = 40\text{--}44$  for *C. sociata* and  $2n = 54$  for *C. uber*. Our results also suggested that *C. tsushimensis* was cytologically distinguished from *C. sociata* and *C. uber*.

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矢野興一<sup>a</sup>, 伊藤久美子<sup>b</sup>, 星野卓二<sup>a, b</sup>: 鹿児島県大隅諸島産カヤツリグサ科スゲ属植物の細胞学的研究 II. 黒島より採集した 4 種の染色体数

大隅諸島黒島より採集したカヤツリグサ科スゲ属植物 4 種について染色体数を報告した. トカラカンスゲ (*Carex atroviridis* var. *scabrocaudata*) が  $2n = 38 = 19II$ ,  $39 = 19II + I$ ,  $58$  であり, 種内異数体が観察された. アオミヤマカンスゲ (*C. multiflora* var. *pallidiquama*) が  $2n = 72$ , フサカンスゲ (*C. tokarensis*) が  $2n = 26 = 13II$ , ツシマスゲ (*C. tsushimensis*) が  $2n = 32 = 16II$  であった. これらの 4 種については今回が初めての報告である. これらのうち, トカラカンスゲとフサカンス

ゲは黒島とトカラ列島に固有である. トカラカンスゲはヒメカンスゲ (*C. conica*) あるいはオオシマカンスゲ (*C. oshimensis*) と細胞学的に近縁であることが明らかとなった. フサカンスゲは近縁種とされているコカンスゲ (*C. reinii*) と同じ染色体数であった.

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